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## Use of the Nematode *Panagrellus redivivus* as an *Artemia* Replacement in a Larval Penaeid Diet

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### Abstract

*Penaeus vannamei* larvae (P<sub>2</sub> through M<sub>3</sub>) and early postlarvae (<24 h old postlarva) were fed diets consisting of algae-only, nematodes (*Panagrellus redivivus*) plus algae or *Artemia* plus algae. Growth (dry biomass gain) of second and third stage protozoa larvae fed the nematode-algae diet was significantly better than that of larvae fed the *Artemia*-algae diet. From the first mysis through the postlarval substage (<24 h old), growth of shrimp fed the nematode-algae diet equalled that of larvae fed the *Artemia*-algae diet. All larval substages fed nematodes plus algae accumulated significantly greater biomass than those fed a diet of only algae. Survival and percent metamorphosis of larvae fed nematodes plus algae did not differ significantly from that of larvae fed either *Artemia* plus algae or algae alone. A nematode-algae feeding regime, which potentially yields growth, survival and metamorphosis equal to that obtained on the standard *Artemia* plus algae regime, is proposed for *P. vannamei*.

A major objective in obtaining predictable production of high quality hatchery reared penaeid postlarvae has been to improve feeding regimes (Wilkenfeld et al. 1981; Qunitio et al. 1983; Wilkenfeld et al. 1984; Kuban et al. 1985). Traditional larval feeding regimes consist of an algal food source at the onset of the protozoal stage followed by an animal food source beginning at or slightly before the mysis stage (Hudinaga and Kittaka 1966; Cook and Murphy 1969; Liao 1984). Due to availability, ease of hatching, and previous success, *Artemia* has become the animal food source of choice in many aquaculture operations (Mock and Murphy 1970; Sorgeloos 1980). However, *Artemia* require additional hatching facilities, are inconsistent in hatchability and nutritional quality, grow too quickly and consume algae in the larval rearing tanks, and can be expensive (U.S. \$15–30 per 454 g of cysts). For these reasons other animal food sources are being investigated.

Use of the nematode *Panagrellus redivivus* (T. Goodey) in penaeid larviculture of-

fers many practical advantages over *Artemia* nauplii. For example, nematodes do not consume algae, can survive for over 72 h in sea water (Fontaine et al. 1982), and never grow too large to be consumed by shrimp larvae (Wilkenfeld et al. 1984). In addition, the ease of culture and the high protein content (48%), have made nematodes a viable food source in the rearing of larval fish for many years (Kahan et al. 1980). Samocha and Lewinsohn (1977) first reported the use of *P. redivivus* in the culture of shrimp. Fontaine et al. (1982) experimented with mass culture methods and different media for growing the nematode and obtained limited success in rearing penaeid larvae fed a nematode-algae diet. Wilkenfeld et al. (1984) suggested that nematodes could partially or completely replace *Artemia* nauplii in the diet of some penaeid species without reducing growth, survival or metamorphic rate. Nematodes have been used on a limited basis in some commercial larviculture facilities; however, nematode levels required by larvae for good growth are not well defined.

This study evaluated various *P. redivivus* feeding levels for larval *Penaeus vannamei*

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(Boone) from the second protozoal through the first postlarval substage. The objective was to determine whether nematodes, when presented in sufficient quantities, could completely replace *Artemia* nauplii in a standard penaeid larval diet. Response to dietary levels was evaluated on the basis of percent survival, percent metamorphosis and accumulated dry weight.

### Materials and Methods

*P. redivivus* were cultured on a medium modified after Wilkenfeld et al. (1984), consisting of 50% (w/w) Masa Harina<sup>1</sup> corn meal and 50% unbleached white wheat flour, mixed with deionized water to the consistency of a soft dough (0.95 mL/g of dry media). Nematodes were cultured on 1.5 cm deep "carpets" of medium in 32 × 25 × 9 cm plastic boxes with lids that were vented with a 4.5 × 13 cm screened (100  $\mu$ ) opening to prevent the entrance of insects. Cultures were maintained in subdued lighting at 25–28 C and were lightly misted daily with deionized water to replace water lost through evaporation.

Each culture was inoculated with approximately 1 million nematodes. Only 5 day old cultures were harvested for use throughout each experiment in order to assure uniform size and nutritional quality of the nematodes. Nematodes were harvested by placing Kimwipes<sup>®</sup> over the medium surface for 30 min and then rinsing adhering nematodes into deionized water. The collected nematodes were concentrated in a separatory funnel, counted and fed live to the larvae following the methods described by Wilkenfeld et al. (1984).

*Artemia* cysts (Biomarine brand,<sup>1</sup> China strain, cyst size 250  $\mu$ , lot #692415) were hatched in aerated, artificial seawater at a salinity of 30 ppt and temperature of 28 C, and were fed to the larvae as instar I or II nauplii (approximate size 515  $\mu$ ). Unialgal

cultures of *Chaetoceros gracile* (Schütt) and *Tetraselmis chuii* (Butcher) were grown in aerated natural seawater enriched with f/2 nutrients using modifications of procedures described by Guillard (1975). Environmental conditions for algal cultures were: temperature 21–23 C, salinity 28–30 ppt, pH 7.8–8.2. Cultures were sampled for use while still in the log phase of growth.

*Penaeus vannamei* larvae were obtained as fourth or fifth stage nauplii from Continental Fisheries, Ltd. (Panama City, Florida). Nauplii were spawned in natural seawater from unilaterally ablated females from Guatemala. Each experiment utilized nauplii from an individual spawn. Nauplii were acclimated to and maintained in artificial seawater (hW-Marine Mix plus bioelements<sup>1,2</sup>), at a density of  $\leq 100/L$ , to which was added algae and 10 mg/L EDTA (ethylenedinitrotetraacetic acid, disodium salt) until the initiation of an experiment. Upon metamorphosis into the first protozoal substage (P<sub>1</sub>), the larvae were stocked at a density of 100 larvae/L into 1 L plastic Imhoff settling cones containing fresh artificial seawater-algae-EDTA mixture (see Wilkenfeld et al. 1983 for details of the system). The cones were held in water baths which maintained temperature in the cones at  $29.9 \pm 0.8$  C. Salinity was maintained at  $30 \pm 1$  ppt, and pH averaged  $8.22 \pm 0.33$ .

Five experiments were performed to determine a feeding density of nematodes for each substage (protozoa two to <24 h old postlarva) which would yield growth, survival and rate of metamorphosis equal to that of larvae fed *Artemia* nauplii. The dietary treatments consisted of an algae-only control (100,000 cells/mL of *C. gracile* plus 30,000 cells/mL *T. chuii*), an *Artemia*-algae diet (the above algal regime plus *Artemia* nauplii), and treatments consisting of algae plus various levels of nematodes (Table 1). For each substage, five replicates were ran-

<sup>1</sup> Use of trade names does not imply endorsement.

<sup>2</sup> Produced for Hawaiian Marine Imports, Inc., by Weigandt GMBH and Co. Kg. D-4150 Krefeld, West Germany.

TABLE 1. Quantity of *Panagrellus redivivus* and *Artemia* nauplii presented to substages of *Penaeus vannamei*. All treatments received 100,000 cells/mL of *Chaetoceros gracile* and 30,000 cells/mL of *Tetraselmis chuii*.

Substage	Experiment	Quantities of nematodes evaluated for each substage (number/mL)	<i>Artemia</i> (number/mL)
P <sub>2</sub>	1	0.0, 5.0, 12.5, <sup>a</sup> 25.0	0.50
	2	0.0, 2.0, 25.0, <sup>a</sup> 40.0	0.50
	3-5	0.0, 5.0 <sup>b</sup>	0.75
P <sub>3</sub>	1	0.0, 10.0, 25.0, <sup>a</sup> 50.0	1.00
	2	0.0, 5.0, 50.0, <sup>a</sup> 80.0	1.00
	3-5	0.0, 10.0 <sup>b</sup>	1.50
M <sub>1</sub>	1	0.0, 15.0, 50.0, <sup>a</sup> 100.0	2.50
	2	0.0, 10.0, 50.0, <sup>a</sup> 150.0	2.50
	3	0.0, 10.0 <sup>b</sup>	2.50
	4-5	0.0, 10.0 <sup>b</sup>	3.00
M <sub>2</sub>	1	0.0, 25.0, 75.0, <sup>a</sup> 150.0	3.00
	2	0.0, 15.0, 75.0, <sup>a</sup> 200.0	3.00
	3	0.0, 25.0, 50.0, 75.0 <sup>a</sup>	3.00
	4-5	0.0, 25.0 <sup>b</sup>	4.00
M <sub>3</sub>	1	0.0, 40.0, 100.0, <sup>a</sup> 200.0	4.00
	2	0.0, 25.0, 200.0, <sup>a</sup> 300.0	4.00
	3	0.0, 25.0, 50.0, 75.0, <sup>a</sup> 100.0	4.00
	4-5	0.0, 25.0 <sup>b</sup>	4.00
PL <sub>0</sub> (<24 h)	1	0.0, 60.0, 125.0, 250.0	5.00
	2	0.0, 125.0, 375.0, 500.0	5.00
	3	0.0, 60.0, 90.0, 125.0, 190.0, 250.0, 375.0	5.00
	4	0.0, 125.0, 375.0, 500.0	5.00
	5	0.0, 35.0, 50.0, 75.0, 125.0	5.00

<sup>a</sup> Level presented to larvae in replicates which were continued beyond this substage (see text).

<sup>b</sup> Level defined in experiments 1-2 or 1-3 (see text).

domly assigned to each dietary treatment (a flow chart which details the stocking, feeding and terminating of the experiments is presented in Fig. 1). The experiment was initiated when nematodes or *Artemia* were added to the appropriate cones upon larval metamorphosis into the protozoa-two substage. Nematode-fed larvae being carried over for later substage evaluations were fed a density of nematodes ("X" in Fig. 1, "a" in Table 1) thought to provide adequate nutrition for growth. Larvae designated for algae-only or *Artemia*-algae diets were fed the same level (Table 1) regardless of termination date. A new seawater-algae mixture was prepared daily following methods described by Wilkenfeld et al. (1983) and utilized in a 100% water exchange to remove metabolic wastes and left over animal feed. New animal feeds were fed at the appropriate levels following the water exchange. Levels of *Artemia* were increased for the

early substages in the last three experiments due to heavier consumption by these groups of animals.

Beginning at the protozoa-three substage, larvae from five predetermined replicates of each treatment were terminated when 90% of the larvae or postlarvae in any of the nematode-algae treatments had metamorphosed into the next substage (Fig. 1). Terminated larvae were counted for survival, staged for metamorphosis, and dried to constant weight at 60 C. Average dry weight/larva, survival, and metamorphic percentage at the terminated substage were used to evaluate the feeding regimes utilized during the preceding substage.

The levels of nematodes required during the protozoa-two (P<sub>2</sub>), protozoa-three (P<sub>3</sub>), and mysis-one (M<sub>1</sub>) substages were defined in the first and second experiments ("b" in Table 1) and were used to feed these three substages in subsequent experiments. The

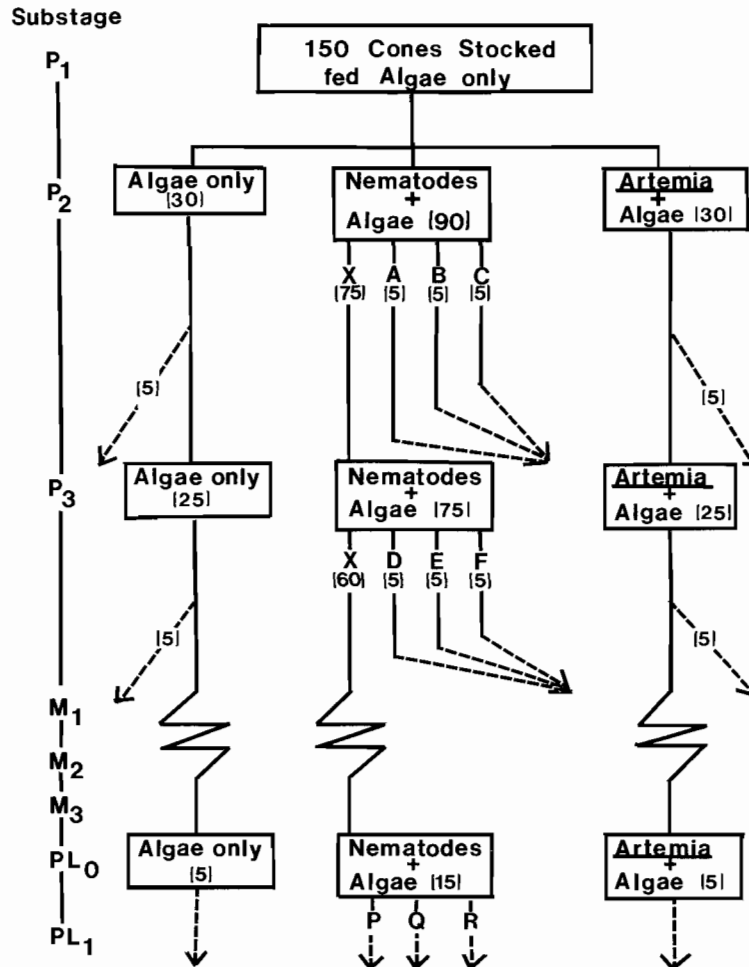


FIGURE 1. Flow chart of an example experimental design. Numbers in parentheses refer to number of replicates. Capital letters represent nematode densities fed. X = levels of nematodes fed to remaining larvae in replicates not being examined at that substage and corresponds to "a" in Table 1. Dashed arrows represent terminations.

third experiment defined levels of nematodes required during the mysis-two (M<sub>2</sub>) and mysis-three (M<sub>3</sub>) substages. The fourth and fifth experiments examined levels during the <24 h old postlarval substage (PL<sub>0</sub>) only, using all previously defined levels for the initial protozoa and mysis substages.

Biochemical analyses of the *Artemia* and nematodes used in the experiments were performed. Total protein was determined using the micro-Kjeldahl method described by Ma and Zuazaga (1942). Total lipid and carbohydrate were determined according to methods of Bligh and Dyer (1959) and Du-

bois et al. (1956), respectively. Lipids were further analyzed for fatty acid content by the Texas A&M Shrimp Mariculture Project physiology laboratory, utilizing gas chromatography methods described by Alam et al. (manuscript in preparation).

Data were analyzed by one way Analysis of Variance and Student Newman Kuels test using SAS software (SAS Institute Inc. 1985). Differences among means were analyzed for significance at the 5% level. Survival and metamorphic percentage data were normalized by arcsine transformation prior to statistical analysis.

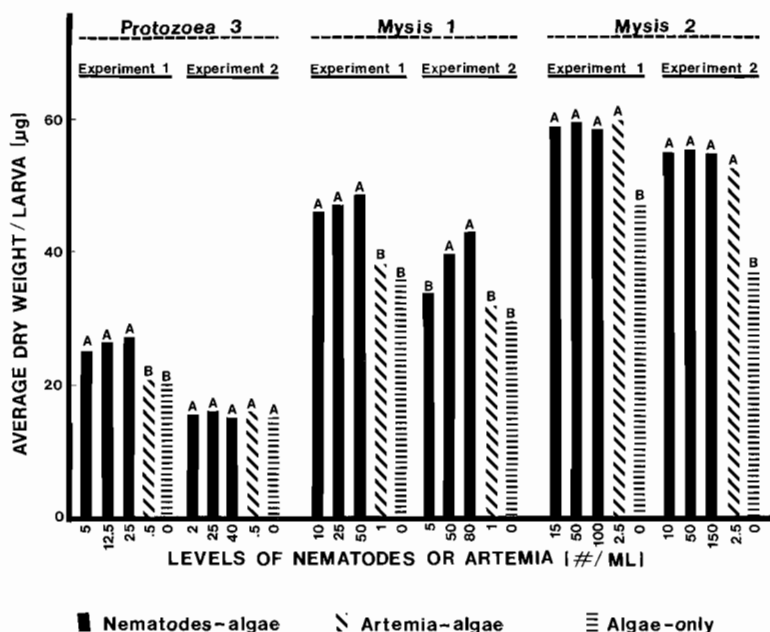


FIGURE 2. Average dry weight of protozoa-three, mysis-one and mysis-two substage larvae (i.e. growth during  $P_2$ ,  $P_3$ , and  $M_1$  substage) fed algae, nematodes plus algae or *Artemia* plus algae. Algae levels were identical in each treatment at 100,000 cells/mL *Chaetoceros gracile* and 30,000 cells/mL *Tetraselmis chuii*. Means within experiments with identical letters are not significantly different (SNK  $\alpha = 0.05$  N = 5).

### Results

No significant differences in percent survival (73.0–88.0%) or metamorphosis (85.5–98.5%) were found among dietary treatments within any of the substages of *P. vannamei*. During the  $P_2$  substage (i.e. fed at  $P_2$  and terminated at  $P_3$ ), significantly better growth was obtained in the first experiment by those larvae fed all levels of nematodes with algae relative to those fed *Artemia* with algae or only algae (Fig. 2). In the second experiment, the addition of nematodes or *Artemia* did not significantly increase the growth of the  $P_2$  substage larvae as compared to those larvae fed only algae (Fig. 2).

During the  $P_3$  substage (i.e. fed at  $P_3$ , terminated at  $M_1$ ), all nematode levels in excess of 5.0 nematodes/mL in experiments 1 and 2 produced significantly better growth than *Artemia*-fed and algae-fed treatments; but, a nematode level of 5.0/mL in the second experiment yielded growth nearly equal to that of larvae which were not fed nematodes (Fig. 2). During the  $M_1$  substage, all

levels of nematodes investigated produced a final dry weight equal to that of *Artemia*-fed larvae and significantly better than that of larvae fed only algae (Fig. 2).

During the  $M_2$  and  $M_3$  substages (i.e. fed at  $M_2$  and  $M_3$ , respectively, and terminated at  $M_3$  and  $PL_0$ , respectively), larvae fed nematodes at levels of 25/mL and above yielded growth equal or better to that of *Artemia*-fed larvae (Fig. 3). Larvae fed *Artemia*, in general, grew better than those fed algae; while larvae fed nematodes consistently showed better growth than larvae fed algae.

The results of the postlarva-one (24 h old) substage termination were variable (Fig. 4). With the exception of experiment 2, dry weight of postlarvae fed a minimum of 125.0 nematodes/mL was generally equal to or significantly greater than that of larvae fed *Artemia*, and consistently greater than that of larvae fed only algae.

In general, larvae fed the nematode-algae treatments outperformed those fed algae-only treatments during all substages and *Ar-*

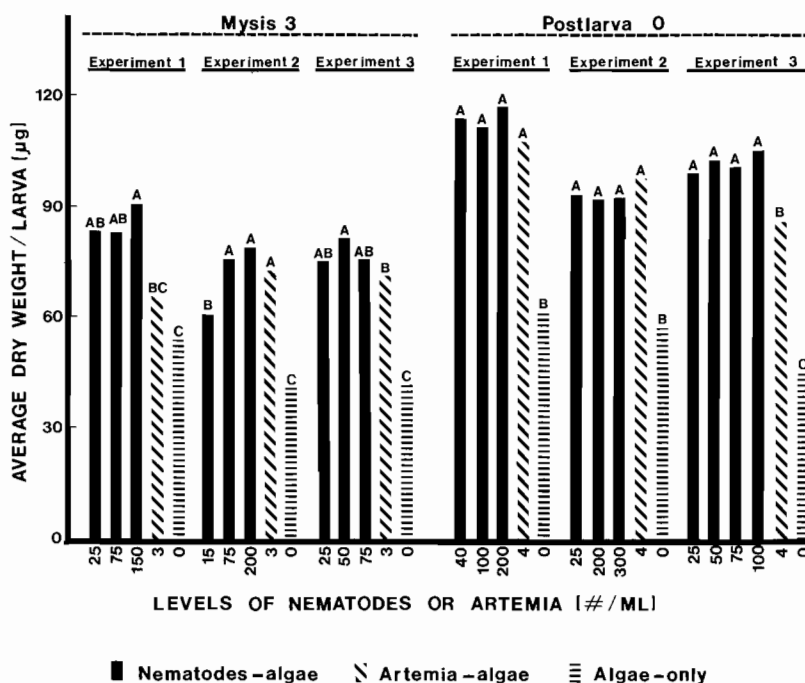


FIGURE 3. Average dry weight of mysis-three and <24 h old postlarva ( $PL_0$ ) substage larvae (i.e. growth during  $M_2$  and  $M_3$  substage) fed algae, nematodes plus algae or Artemia plus algae. Algae levels were identical in each treatment at 100,000 cells/mL *Chaetoceros gracile* and 30,000 cells/mL *Tetraselmis chuii*. Means within experiments with identical letters are not significantly different (SNK  $\alpha = 0.05$  N = 5).

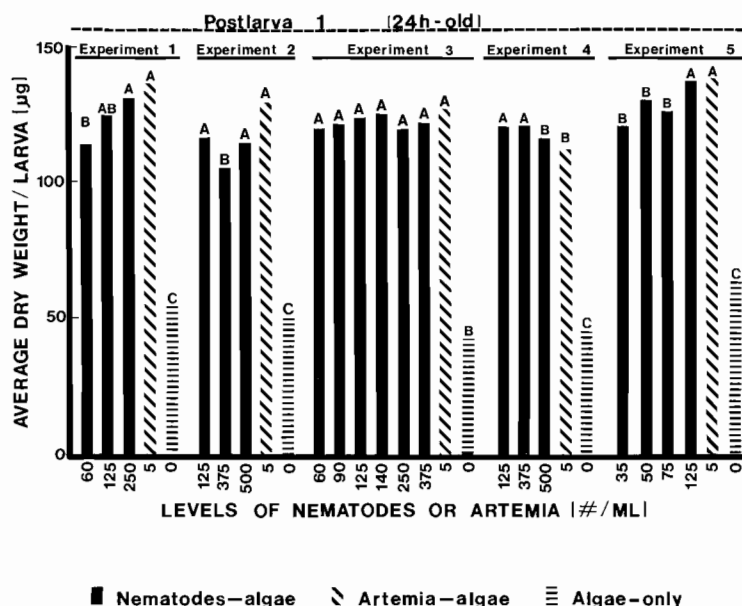


FIGURE 4. Average dry weight of 24 h old postlarva substage larvae (i.e. growth during  $PL_0$ ) fed algae, nematodes plus algae or Artemia plus algae. Algae levels were identical in each treatment at 100,000 cells/mL *Chaetoceros gracile* and 30,000 cells/mL *Tetraselmis chuii*. Means within experiments with identical letters are not significantly different (SNK  $\alpha = 0.05$  N = 5).

TABLE 2. Biochemical analyses of 1st and 2nd instar *Artemia* and 5-day old cultures of *Panagrellus redivivus*. Values are in % dry weight  $\pm$  standard deviation. Means with identical letters are not significantly different (SNK  $\alpha = 0.05$  N = 3).

	Protein	Lipid	Carbohydrate
<i>Artemia</i>	59.5 $\pm$ 1.0 A	18.6 $\pm$ 1.0 A	17.7 $\pm$ 0.6 A
<i>P. redivivus</i>	48.3 $\pm$ 2.2 B	17.3 $\pm$ 0.4 A	31.3 $\pm$ 2.2 B

*temia*-algae treatments during both the P<sub>2</sub> and P<sub>3</sub> substages. Growth of larvae fed the *Artemia*-algae diet did not differ significantly from larvae fed only algae during the protozoal stages. Growth of larvae fed the nematode-algae diet equalled or surpassed that of larvae fed *Artemia* during the M<sub>1</sub> through PL substages, while growth of larvae fed either nematodes or *Artemia* surpassed that of larvae fed algae-only.

Results of the biochemical analyses revealed a significantly lower level of protein in nematode tissue (48%) than in *Artemia* tissue (58%). Nematodes had a significantly higher carbohydrate level, which was three times that of *Artemia*, while lipid levels were not significantly different between the two sources (Table 2). Fatty acid analysis of the lipid fraction (Table 3) revealed that *Artemia* contained a greater percentage of myristic (14:0), palmitic (16:0), palmitoleic (16:

1n-7), linolenic (18:3n-3), arachidic (20:0) and eicosapentaenoic (20:5n-3), while nematodes contained a greater percentage of steric (18:0), oleic (18:1n-9), linoleic (18:2n-6), nonadecyclic (19:0), heneicosanoic (21:0), arachidonic (20:4n-6), and docosahexenoic (22:6n-3) acids.

### Discussion

The experimental data suggests that the addition of relatively small densities of nematodes to an algal diet will suffice as an animal food source for *P. vannamei* larvae through the M<sub>3</sub> substage. Apparently the dramatic increase in growth associated with metamorphosis into the PL substages requires a considerable increase in the density of nematodes. An estimate of the minimum level of nematodes needed at each larval substage, in conjunction with algae, to obtain growth equal to that achieved with densities of algae and *Artemia* are given in Table 4.

Examination of the protozoal substages suggests that growth benefitted more from

TABLE 3. Fatty acid profiles of 1st and 2nd instar *Artemia* and 5-day old cultures of *Panagrellus redivivus*. Values represent % of total fatty acids.

Fatty acid	<i>Artemia</i> (%)	Nematodes (%)
14:0	6.261	0.549
16:0	9.902	8.538
16:1n-7	12.964	2.708
17:0	0.468	1.376
18:0	2.529	5.341
18:1n-9	18.145	19.144
18:2n-6	3.969	36.104
18:3n-3	3.031	1.729
19:0	0.721	1.111
20:0	1.978	0.826
20:2n-6	0.358	3.477
20:4n-6	1.504	4.534
20:5n-3	7.219	2.828
21:0	5.190	4.134
22:6n-3	0.078	0.195
Unidentified	25.683	7.406

TABLE 4. Estimated minimum levels of nematodes to be fed in conjunction with algae to yield growth equal to that obtained with an *Artemia*-algae diet. Algal densities are identical in both regimes at 100,000 cells/mL *Chaetoceros gracile* and 30,000 cells/mL of *Tetraselmis chuii*.

Substage	Nematodes (number/mL)	<i>Artemia</i> (number/mL)
P <sub>2</sub>	5.0	0.5
P <sub>3</sub>	10.0	1.0
M <sub>1</sub>	10.0	2.5
M <sub>2</sub>	25.0	3.0
M <sub>3</sub>	25.0	4.0
PL <sub>0</sub> (<24 h old)	125.0	5.0



TABLE 5. Amino acid profiles of *Panagrellus* spp., *Artemia* and *Penaeus japonicus* larvae. Values represent weight percent of total amino acids.

Amino acid	<i>Panagrellus</i> spp. <sup>a</sup> (%)	<i>Artemia</i> spp. <sup>b</sup> (%)	<i>P. japonicus</i> <sup>c</sup> (%)	EAA <sup>d</sup>
Lysine	7.9 ± 0.6	8.5	8.4	+
Histidine	2.9 ± 0.2	2.3	2.9	+
Arginine	6.6 ± 0.4	7.1	9.1	+
Aspartic acid	11.2 ± 0.3	10.5	11.4	
Threonine	4.7 ± 0.8	4.8	3.8	+
Serine	3.7 ± 0.9	6.4	6.4	
Glutamic acid	12.8 ± 1.7	13.6	15.6	
Proline	5.4 ± 0.2	5.9	6.6	
Glycine	6.4 ± 0.2	4.8	5.8	
Alanine	8.8 ± 0.1	5.5	6.2	
Cysteine	0.0	0.0	1.5	
Valine	6.4 ± 0.2	5.2	5.6	+
Methionine	2.2 ± 0.6	2.3	3.7	+
Isoleucine	5.1 ± 0.2	4.4	5.9	+
Leucine	7.7 ± 0.1	7.6	7.8	+
Tyrosine	3.2 ± 0.5	4.4	5.9	
Phenylalanine	4.9 ± 0.2	4.2	5.6	+
Tryptophan	— <sup>e</sup>	— <sup>e</sup>	4.0	

<sup>a</sup> From Kahan et al. (1980).

<sup>b</sup> From Appelbaum (1979) cited in Kahan et al. (1980).

<sup>c</sup> Analysis performed on a combination of protozoal substages in Teshima et al. (1986).

<sup>d</sup> Essential Amino Acids demonstrated by Cowey and Forster (1971) and Shewbart et al. (1972).

<sup>e</sup> Tryptophan was destroyed in the analytical process.

the addition of dietary nematodes than from the addition of *Artemia*. This observation may be explained by the ability of the early larval substages to more easily capture the nematodes, which are smaller than the *Artemia*. Even though penaeid larvae consume *Artemia* as early as the P<sub>2</sub> substage (Wilkenfeld et al. 1984; Kuban et al. 1985), in commercial applications, *Artemia* are rarely added until the late P<sub>3</sub> or early M<sub>1</sub> stage. Since 100% water exchanges are rarely performed in commercial situations, uneaten *Artemia* may compete with the larvae for algae, thus limiting algal availability to protozoa. Unlike *Artemia*, nematodes do not consume algae.

Previous studies investigating nematodes as food for penaeid larvae have had mixed results, possibly due to the low levels of

nematodes used. For example, Fontaine et al. (1982) obtained variable survival of *P. stylirostris* using frozen nematodes at a static survival of 30/mL. Wilkenfeld et al. (1984) obtained better survival when larval penaeids were fed live nematodes at a static level of 70/mL, disproving Fontaine's previously reported explanation that penaeid larvae could not capture and consume live nematodes. Wilkenfeld et al. (1984) also recognized the importance of growth as a key indicator of the performance of a diet. They suggested that for penaeid species more sensitive to the presence of animal food, such as *P. vannamei*, or for feeding regimes containing an inferior algal source, diets containing *Artemia* consistently produced greater growth to postlarvae than diets containing static levels of nematodes. In contrast, the present data suggest that in the presence of sufficient quantities of nematodes with algae, final biomasses of *P. vannamei* postlarvae can equal those obtained on a diet of *Artemia* with algae.

Biochemical analysis of nematodes and *Artemia* revealed that both contained relatively high levels of protein and lipid (Table 2). Absolute nutritional requirements of penaeid larvae are not known, although relatively high levels of protein have been found to be important in the growth of the early postlarval stages of *P. stylirostris* and *P. californiensis*, suggesting that larval protein requirements may be equal to or greater than 44% (Colvin and Brand 1977). Further analysis of the protein of *Panagrellus* spp. by Kahan et al. (1980) revealed that the amino acid profile was similar to that of *Artemia* (Table 5). An amino acid profile of *P. vannamei* larvae is not available in the literature; however, the profile of larval *P. japonicus* reported by Teshima et al. (1986) reveals similar amino acid content to both nematodes and *Artemia* especially in those amino acids thought to be essential in the diets of shrimp (Cowey and Forster 1971, Shewbart et al. 1972). Carbohydrate sources in the diet are also thought to be important for sparing amino acid sources during chitin

synthesis (Cowey and Forster 1971). Molting occurs frequently during larval growth and may quickly diminish the available carbohydrate pool (Dall 1965).

Fatty acid analyses of nematodes and *Artemia* revealed several differences in lipid composition (Table 3). Fatty acid requirements and the nature of fatty acid metabolism in penaeid larvae are not fully understood. Ward et al. (1979) determined that the major fatty acids of the egg and nauplius of *P. setiferus* which remain major fatty acids in the postlarval stage were 16:0, 18:0, 18:1n-9, 20:4n-6, and 20:5n-6 and postulated that these fatty acids appear to be required in larger quantities than the other fatty acids. Other studies have provided strong evidence that highly unsaturated fatty acids, especially 20:5n-3 and 22:6n-3, play an important role in penaeid larval nutrition and may be required in larval diets (Jones et al. 1979). Nematodes, although seemingly lacking in 20:5n-3 in comparison to *Artemia*, contain a higher percentage of arachidonic acid (20:4n-6), while neither nematodes nor *Artemia* contain very high levels of 22:6n-3. Under these experimental conditions, contributions from the algae may have corrected possible (potential) fatty acid deficiencies in either of the feeding regimes.

Ultimately, successful use of the nematode *P. redivivus* in large scale larviculture of penaeids will depend on several factors. The ability and the space available to culture and maintain nematodes in mass quantities would be the first requirement. Successful small scale mass culturing has been achieved during the performance of this investigation, with production of up to 6,000 nematodes/cm<sup>2</sup> of medium. Fontaine et al. (1982) reported culturing up to 11,000 nematodes/cm<sup>2</sup> on an oatmeal medium. Other studies suggest that the addition of glucose or saltwater to the medium may increase the production and growth of nematodes (Buecher et al. 1970; Kahan and Appel 1975). Currently, mass culturing the quantities of nematodes needed at the later substages for penaeid larviculture to obtain

growth equal to that of *Artemia* is the major limiting factor for commercial applications.

Another consideration is the maintenance of water quality. Water quality may be reduced enough to cause mortalities if precautions are not taken during the harvesting of nematodes to minimize the introduction of extraneous medium into the larval rearing tank. Additionally, because nematodes are even less buoyant than *Artemia*, aeration may have to be increased or optimized to prevent nematodes from settling out of the water column. Regular water exchanges must also be performed to maintain water quality, due to the death and decay of nematodes after 72 h in salt water. Finally, operative costs for medium and labor must be considered.

Nematodes appear to be a promising food source for commercial penaeid larviculture, but further research is needed on mass culture methodology to make their use cost effective.

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## Literature Cited

- Alam, N., F. L. Castille and A. L. Lawrence. (manuscript in preparation). Fatty acid analysis of shrimp tissues by gas chromatography. Shrimp Mariculture Project. Texas Agricultural Experiment Station Texas A&M University, Port Aransas, Texas, USA.
- Appelbaum, S. 1979. The suitability of alkan-yeast (hydrocarbon grown yeast) as a first nutrient for *Coregonus albula* (L.) fry. Pages 515-524 in J. E. Halver and K. Tiews, editors. Finfish nutrition and fishfeed technology, volume 1. Proceeding of a world symposium, Hamburg, West Germany, June 1978. Schr. Bundesforschungsanst. Fisch.
- Bligh, E. G. and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911-917.
- Buecher, E. J., E. L. Hansen and T. Gottfried. 1970. A nematode growth factor from Baker's yeast. Journal of Nematology 2:93-98.
- Colvin, L. B. and C. W. Brand. 1977. The protein requirement of penaeid shrimp at various life-cycle stages in controlled environment systems. Proceedings of the World Mariculture Society 8:821-840.
- Cook, H. L. and M. A. Murphy. 1969. The culture of larval penaeid shrimp. Transactions of the American Fisheries Society 98:751-754.
- Cowey, C. B. and J. R. M. Forster. 1971. The essential amino acid requirements of the prawn *Palaeomon serratus*. The growth of prawns on diets containing proteins of different amino acid compositions. Marine Biology 10:77-81.
- Dall, W. 1965. Studies on the physiology of a shrimp, *Metapenaeus* sp. (Crustacea: Decapoda: Penaeidae) IV. Carbohydrate metabolism. Australian Journal of Marine and Freshwater Resources 16: 163-180.
- Dubois, M., K. A. Gilles, J. K. Hamilton, B. A. Rebers and F. Smith. 1956. Colorimetric method for the determination of sugars and related substances. Analytical Chemistry 28:350-356.
- Fontaine, C. T., D. B. Revera, H. M. Morales and C. R. Mock. 1982. Observations on the mass propagation of the common soil nematode *Panagrellus redivivus*, for use as foodstuff in penaeid shrimp hatcheries. NOAA Technical Report, National Marine Fisheries Service, Galveston Laboratory, Galveston, Texas, USA.
- Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates. Pages 29-60 in W. L. Smith and M. H. Chanley, editors. Culture of marine invertebrate animals. Plenum Publishing Corporation, New York, New York, USA.
- Hudinaga, M. and J. Kittaka. 1966. Studies on food and growth of larval stage prawn, *Penaeus japonicus* Bate. Informational Bulletin on Planktology, Japan 13:83-94.
- Jones, D. A., A. Kanazawa and K. Ono. 1979. Studies on the nutritional requirements of the larval stages of *Penaeus japonicus* using micro-encapsulated diets. Marine Biology 54:261-267.
- Kahan, D. and Z. Appel. 1975. The value of *Panagrellus* sp. (Nematoda) as food for fish. 10th European Symposium on Marine Biology, Ostend, Belgium, Sept. 17-23, 1975. 1:243-253.
- Kahan, D., T. Bar-El, Y. Brandstein, M. Rigbi and B. Oland. 1980. Free living nematodes as a dietary supplement in the rearing of fish fry and hatcheries. General Fisheries Council Mediterranean Studies Review 57:67-78.
- Kuban, F. D., A. L. Lawrence and J. S. Wilkenfeld. 1985. Survival, metamorphosis and growth of larvae from four penaeid species fed six food combinations. Aquaculture 47:151-162.
- Liao, I. C. 1984. A brief review of the larval rearing techniques of penaeid prawns. Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps 1:65-78.
- Ma, P. S. and G. Zuazaga. 1942. Micro Kjeldahl determination of nitrogen. A new indicator and an improved rapid method. Industrial and Engineering Chemistry 14:280-282.
- Mock, C. R. and M. A. Murphy. 1970. Techniques for raising penaeid shrimp from egg to postlarvae. Proceedings of the World Mariculture Society 1:143-156.
- Quinitio, E., D. de la Pena and F. Pascual. 1983. The use of suitable feeds in larval rearing of *Penaeus monodon*. Pages 337-342 in G. L. Rogers, R. Day and A. Lim, editors. Proceedings of the First International Conference on Warm Water Aquaculture-Crustacea. Brigham Young University Hawaii Campus, Honolulu, Hawaii, USA.
- Samocha, T. and C. H. Lewinsohn. 1977. A preliminary report on rearing penaeid shrimps in Israel. Aquaculture 10:291-292.
- SAS Institute Inc. 1985. SAS/STAT Guide to Personal Computers. Version 6. Cary, North Carolina, USA.
- Shewbart, K. L., W. L. Mies and P. D. Ludwig. 1972. Identification and quantitative analysis of the amino acids present in protein of the brown shrimp, *Penaeus aztecus*. Marine Biology 16:64-67.
- Sorgeloos, P. 1980. The use of the brine shrimp *Artemia* in aquaculture. Pages 25-46 in G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers, editors. The brine shrimp *Artemia*, volume 3. Ecology, culture, use in aquaculture. Universal Press, Wetteren, Belgium.
- Teshima, S., A. Kanazawa and M. Kamashita. 1986. Dietary value of several proteins and supplemental amino acids for larvae of the prawn *Penaeus japonicus*. Aquaculture 51:225-235.
- Ward, D. G., B. S. Middleditch, S. A. Missler and A.

- L. Lawrence.** 1979. Fatty acid changes during larval development of *Penaeus setiferus*. Proceedings of the World Mariculture Society 10:464-471.
- Wilkenfeld, J. S., J. M. Fox and A. L. Lawrence.** 1981. Consumption of frozen and live *Artemia* by protozoa of *Penaeus setiferus*. Journal of the World Mariculture Society 12(2):250-254.
- Wilkenfeld, J. S., A. L. Lawrence and F. K. Kuban.** 1983. Rearing penaeid shrimp larvae in a small scale system for experimental purposes. Pages 72-81 in G. L. Rogers, R. Day and A. Lim, editors. Proceedings of the First International Conference on Warm Water Aquaculture-Crustacea. Brigham Young University Hawaii Campus, Honolulu, Hawaii, USA.
- Wilkenfeld, J. S., A. L. Lawrence and F. D. Kuban.** 1984. Survival, metamorphosis, and growth of penaeid shrimp larvae reared on a variety of algal and animal foods. Journal of the World Mariculture Society 15:31-49.